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BIOELECTROCHEMISTRY II: MEMBRANE PHENOMENA(U) COLUMBIA  
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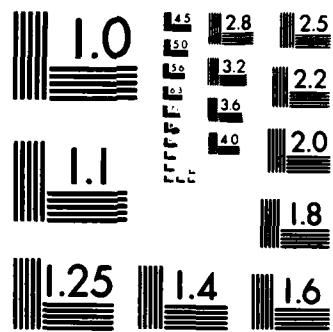
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DEPARTMENT OF PHYSIOLOGY

630 West 168th Street

11 December 1984

Dr. Michael Marron  
Biological Science Division  
Office of Naval Research  
800 North Quincy Street  
Arlington, VA 22217

RE: N00014-84-G-0183

**AD-A149 132**

Dear Dr. Marron:

I am enclosing five copies of the report on our meeting in Erice, Italy. As you can see from the summary, the meeting went well and the discussions were lively.

We would like to thank you again for your support.

Sincerely yours,

*D. Schachter*

D. Schachter, MD  
Principal Investigator

cc: ONR Res. Rep/G.R. Bellisari  
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A REPORT  
BIOELECTROCHEMISTRY II: MEMBRANE PHENOMENA

The second advanced course on bioelectrochemistry at the International School of Biophysics took place at the Majorana Center in Erice, Italy, during 5-15 November 1984. Three years ago, the first such meeting, also organized by G. MILAZZO (Rome) and M. BLANK (New York), had been devoted to redox processes, a subject with strong and obvious links to electrochemistry. The subject of this meeting, membrane phenomena, was much broader, but the emphasis was on the electrochemical aspects of ion transport, energy transduction, and signal conduction. In addition, there were discussions on three practical applications of bioelectrochemistry, the effects of applied electric fields on membranes, mediated and non-mediated transport of pharmacologic agents and the exploitation of information about biological membranes in the development of industrial processes.

The 64 participants came from many scientific disciplines including microbiology, physiology, biochemistry, biophysics, botany and physical chemistry, and the discussions reflected this. Of the 18 nationalities represented, one third of the participants were from Italy, and the rest from other European countries including three eastern block nations as well as North America and Asia.

The first major subject considered was the structure and stability of membranes. MILLER's (Rehovot) lecture on membrane models focused on two systems, the unusual bipolar lipids of archbacteria membranes, and the cerebrosides commonly found among brain lipids. The lipids of archbacteria contain cyclopentyl groups that keep the chains fluid, even at low temperatures, and protect the membrane from crystallization. The cerebrosides contain glucose and amide linkages in the polar region of the molecules that retain structure and cause large hysteresis effects. Hysteresis is a physical measure of memory, and the locking of the lipid molecules into unstable conformations could be the molecular basis of memory in the brain. During nerve activity, changes in the electric field across the membrane and local heating effects due to the ionic currents could cause changes in cerebrosides associated with memory.

MILLER also spoke about the physical aspects of ion transport through lipid bilayers. Considering the energetics of pore formation, he showed that a toroidal pore is more likely to form than a cylindrical one. Although the interfacial area (between water and membrane molecules) is greater with a toroidal pore, the interfacial free energy is much lower because the polar groups rather than the  $\text{CH}_2$  groups of the membrane lipids are in contact with the water. The charges at membrane surfaces form electrical double layers that can affect the membrane structure, e.g., a loss of planarity, electrostrictive effects and dielectric breakdown. In studies of the leakage of  $\text{Ca}^{+}$  ions from dipalmitoyl lecithin vesicles, it was possible to show spontaneous formation of transient (lifetimes 60 sec) pores that depend upon the temperature and tonicity of the medium. The vesicles become very much more permeable in the temperature range around the phase transition temperature of the phospholipids.

The second lecture on membrane models was given by CHAPMAN (London) who presented results using several different physical techniques for studying protein-lipid interactions and molecular movements in membranes. He discussed spin labels, fluorescent probes, NMR studies and recent infrared techniques using microprocessors to correct for the water signal. He stressed the importance of using several techniques since the results of any one technique are not always clear. In recent studies of the boundary lipid around membrane proteins, which are not different from other membrane lipids, some techniques show differences. Two practical problems that Chapman discussed were: 1) the protection trehalose provides against membrane damage during dehydration, and 2) the formation of stable liposomes by using diacetylemic phospholipids and polymerizing them once the membrane structures are formed. Both problems are providing useful information about membrane properties.

Moving on to real membranes, DE GIER (Utrecht) discussed the properties of the different classes of amphipathic membrane lipids. There are very clear relations between molecular properties (e.g. polar groups, chain length, the number of double bonds) and important membrane properties (e.g. permeability, phase transition temperature). The mechanism of pore formation (diameters about 8Å) in continuous lipid phases at the transition temperature is quite well characterized from transport studies and the enhanced transfer of lipids between the two layers of a bilayer (flip-flop). Certain molecules (e.g. lysolecithin, membrane protein) also cause an increase in permeability by the formation of defect regions. However, glycoporphin, a major red cell protein, forms a good "seal" with membrane lipids because of the hydrophobic amino acids interacting with the hydrocarbons. The formation of inverted micelles in the lipid structures that show up in electron microscope freeze fracture patterns can result in the enhanced transport of the contents of the inverted phase.

The other major component of natural membranes, protein, exists in many different forms, e.g., enzymes, receptors, ion channels, energy transducing complexes, as well as, structural elements. LENAZ (Bologna) provided an excellent introduction to this broad topic, along with a few well chosen examples of increasing complexity to set the stage for the discussion of transport, energy transduction and electrical excitation. He started with the methods for isolation and reconstitution, ways of determining the sidedness and topology of the molecule in the lipid matrix by the use of crosslinking agents and photoactivation techniques, and went on to lipid protein interactions, protein mobility and the properties of membrane bound enzymes. The first example relating to function was gramicidin, a polypeptide that forms an ion channel as a result of head-to-head dimerization. The inner core of an  $\alpha$  helix is not large enough to allow ions to pass, but gramicidin forms a hydrophilic ion channel with a hydrophobic outer surface to stabilize it within the membrane lipid matrix. An example of a natural channel is bacteriorhodopsin, a single chain of about 25 kilodaltons with 7 helical portions that cross the membrane to form a polar pore that conducts protons in the presence of light. The much more elaborate mitochondrial complex that includes a channel and a phosphorylase is still not adequately characterized. Protein mobility in membranes is generally low, and the times involved in electron transport processes are fast

compared to the times required by protein molecules to make physical contacts as a result of diffusion. Ubiquinone can diffuse fast enough to accomplish this function in mitochondria.

The next major topic was membrane transport. NEUMANN's (Bielefeld) lecture on ionic reactions at membrane surfaces introduced the basic electrochemistry involved when electric fields are applied to membranes, in particular to the membrane proteins. The enzymes, receptors, transport proteins, and channels, within the membrane matrix or at the membrane surfaces, contain and are in the vicinity of charges and dipoles that affect diffusion rates to and along the membrane surfaces as well as reaction rates. Neumann's analysis of the binding of a quaternary nitrogen containing group to a membrane acetylcholine esterase is useful for predicting the changes in binding to the receptor protein (AChR) due to changes in the physical properties of the AChR molecule. This model is also useful for explaining the conductance change due to the binding of acetylcholine.

The next lecture by BERG (Jena) dealt with membrane breakdown and fusion brought about by electric fields. Relatively low intensity electric fields cause the migration and orientation of cells and the formation of "pearl chains", i.e., rows of cells that are in contact with each other. When the cells are touching, a high intensity, short duration pulse can cause the fusion of cell membranes. The high intensity field ruptures the membrane, but the short duration allows the membrane to reform and the two membranes to fuse. Many different cells have been formed by this process, e.g., about 500 erythrocytes have been fused into a single cell, various combinations of gametes (e.g., oocytes) and of hybridomas (i.e., an antibody producing cell with a cancer cell) have been reported. Macro-techniques use many cells simultaneously while micro-techniques use needle electrodes in two cells to achieve a large degree of control over the process.

CAPLAN (Rehovot) lectured on the non-equilibrium thermodynamics of transport. After a brief summary of the basics of classical thermodynamics, he developed the central ideas of entropy production during irreversible processes and the definition of the dissipation function in terms of the flows and driving forces in a system. Applying these ideas to membrane transport, it is possible to delineate the experimental variables that must be measured in order to characterize active transport in epithelia and also to analyze the results of isotope experiments. Caplan gave one example where the improper analysis of tracer flux data led to the erroneous conclusion about magnitudes of the fluxes. The last topic covered was coupling mechanisms and the definition of efficiency. Non-equilibrium thermodynamics offers a useful formalism for the analysis of transport processes and the definition of the components of a system. However, as systems become more complicated, ambiguities can arise, and the discussion at the end of the presentation dealt with a system where there was a disagreement as to whether the equations adequately described the experimental system that was being studied.

LAUGER (Konstanz) next spoke about mediated and non-mediated ion transport through lipid bilayers. He described the differences between a

carrier and a channel and presented data for examples of each type. Valinomycin is a carrier of  $K^+$  ions because the ion interacts with the polar core of the molecules and the hydrophobic exterior can dissolve in the membrane lipids. There is an upper limit of about  $10^5$  ions/sec transported, because of the need for the whole complex to move through the membrane lipid. This can be increased somewhat by using more unsaturated, more fluid lipids. The inner core of the channel forming gramicidin is also polar because of many carbonyl groups, but two gramicidins join to form a continuous polar pathway between the two aqueous phases. The rate of flow through this channel, in the range of  $10^8$  ions/sec, increases with ionic size for the alkali ions and also with temperature. Mixtures of gramicidin with positive or negative groups on the molecules lead to rectification effects. Analysis of the fluctuations in conductance provides information about these systems.

The final talk on the subject of ion transport was given by PASSARELLA (Bari) who had some interesting results on the effects of laser radiation. Applying laser irradiation of 5 joules/cm<sup>2</sup> to mitochondria has no apparent effect on osmotic or enzymatic properties, but there is a clear increase in ATP production that is inhibited by antimycin. Although the mechanism is unknown, during the discussion it was suggested that the irradiation is probably having its effect by converting NADH to NAD<sup>+</sup>.

The next major topic, energy transduction, was introduced by WILSON (Philadelphia), who spoke about mitochondrial oxidative phosphorylation. He described the respiratory chain, the different complexes, the redox potentials of the components, and the energetics of phosphorylation. The respiratory rate, i.e. the reaction with oxygen, is regulated by three factors: NADH/NAD<sup>+</sup>, ATP/ADP and the  $pO_2$ . Mitochondria may function as tissue oxygen sensors and control the reflexes that help adjust blood flow (e.g., in the coronary artery). Cytochrome C oxidase, the enzyme complex that catalyzes the reaction with oxygen, has many components and the reactions have yet to be completely understood.

MELANDRI (Bologna) then discussed the biochemistry of chemiosmosis. Mitchell's hypothesis about the alternation of oppositely directed proton and electron transporting reactions in mitochondria was presented along with a discussion of the reactions during photosynthesis. It is clear that the protonmotive force is composed of differences in chemical potential of the protons ( $\Delta\mu_H$ ) and differences of electrical potential, and that this quantity plays an important role in phosphorylation. However, the quantitative aspects are not clear. The experimental evidence depends upon indirect measurements (e.g., dye distribution, electrochromism, fluorescence). Also, measurements are made over relatively large regions while the relevant processes occur in microscopic domains. Further measurements are obviously needed to work out the details of chemiosmosis.

The third lecture in this group, by GRABER (Berlin), focused on primary charge separation and energy transduction in chloroplasts. He reviewed the components and reactions at the two reaction centers in photosynthesis, and carefully correlated the structure with the

components of thylakoids, their redox potentials and the kinetics of their reactions. Ingenious light gradient experiments leading to the generation of electrochemical potential gradients in thylakoid suspensions, enabled the direct measurement of the charge separation and the determination of rate constants in the nanosecond range. The ATPase has a protein component that couples the charge separation across the ion channel to the enzyme. Experiments on this protein should help to clarify how "coupling" occurs at the molecular level.

The next major topic was the effect of electric signals on membranes. BLANK (New York) first summarized nerve excitation on both cellular and molecular levels, and then considered nerve membranes and ion channels from an electrochemical point of view. When the properties of charged surfaces (i.e., surface concentrations, surface potentials and surface capacitances) are used to describe ion transport in an excitable membrane containing voltage gated channels, it is possible to explain the currents during a voltage clamp. It is also possible to show that the gating current conductance determines the ionic specificity of the channel. Considering aggregation equilibria of oligomeric proteins, such as those found in ion channels, changes in surface charge density brought about by "gating currents" can cause the opening and closing of channels. This idea accounts for the various reactions (e.g., aggregation, oxygenation) of hemoglobin and offers a way of explaining the mechanisms of voltage and ligand gated channels.

CONTI (Camogli) presented the different lines of evidence (i.e., using neurotoxins, membrane noise analysis, measurements of gating currents and patch clamping) that support the discrete nature of the permeability changes in excitable membranes. In squid axons there appear to be about five times as many sodium channels as potassium channels. The numbers of channels differ in different nerves, but the single channel conductance appears to be characteristic for each type of channel. A statistical and thermodynamic analysis of the permeability changes leads to predictions about the behavior of the channel as a function of voltage, temperature and pressure. The data show a decrease in entropy of about  $20k$  and an increase in volume of about  $40 \text{ \AA}^3$  when a channel opens. The decrease in entropy coupled with an increase in volume is unusual and may refer to processes like the freezing of water, in which case the magnitude of the changes implicate 10-15 water molecules.

Before the Round Table sessions, there were a number of short presentations by PIETROBON (Padua) on uncoupling in proton pumps, SMAAL (Utrecht) on phosphatidic acid as a  $\text{Ca}^{2+}$  ionophore, COLOSIMO (Rome) on cytochrome C, SYMONS (Rehovot) on electric field stimulated luminescence, FATO (Bologna) on lateral diffusion of ubiquinone and PETTY (Durham) on practical application of Langmuir Blodgett films. These talks highlighted some of the specific problems that had been discussed earlier and also provided a transition to the more open discussion oriented sessions on practical applications.

KORENSTEIN (Rehovot) chaired the Round Table on mechanistic and kinetic aspects in the interaction of external electric fields with vesicular membranes and integral membrane proteins. The low magnitude of the induced electric field internally can account for the experiments on



dielectric breakdown and piezoelectric effects, but it is difficult to explain the stimulation of cell growth and repair. The biological effects may result from the secretion of growth factors, hormones, second messengers, etc., but the mechanisms are unknown. CONTI discussed some of the effects of high voltages on nerve axon membranes, and BERG showed an inverse relation between vesicle diameter and the electric field strength required for fusion. HARRIS (Aberystwyth) presented some new data on dielectric dispersion indicating more rapid diffusion ( $D = 10^{-7}$  cm<sup>2</sup>/s) of proteins in membranes than has been reported, and BLANK showed similar data for proteins in newly formed nerve membranes. MARKOV (Sofia) indicated that low magnetic fields can affect cell membranes, and GRATTAROLA (Genoa) had data to suggest that bone healing could be due to the modification of surface receptors by microelectrophoresis or aggregation.

The Round Table on mediated and non-mediated transport through biomembranes of materials of pharmacological interest was chaired by METZNER (Tubingen), who opened the session with a review of the many transport mechanisms (e.g. passive, facilitated, active, pinocytosis) found in cells. Some algae can enrich Cr and Mn by factors of  $10^{11}$  over the external concentration, and many mechanisms discriminate between d and l forms of molecules. SALMONA (Milan) described the factors that control the concentration of a drug at different sites in the body. Differences in absorption, perfusion, partition and diffusion in tissues as well as the possibility of extra barriers in diseased states make it difficult to predict the time course of the concentration of a drug and its metabolites. There are also problems in the design of a drug which has to be hydrophobic to penetrate membranes but hydrophilic to be excreted after it is partially metabolized. Many examples illustrated these points. MASTURZO (Milan) described how membrane fluidity can be modulated by drugs. BERG discussed the mechanics of one group of cytotoxic drugs, the anthracyclines, and described how changes in the molecule affect metabolic pathways and membrane transport. The best hope for insuring action at targeted organs appears to be labeled liposomes.

The last discussion session entitled "From Biological Membranes to Industrial Processes" was introduced by BUVET (Creteil), who reviewed the current state of membrane technology. Biological evolution has led to very efficient ion transport and energy transduction processes in natural membranes, but industry need not be constrained by biological solutions. In thylakoid membranes, the recombination of charged species formed from the splitting of water by light is avoided by cascades of catalysts, but engineers can also use inorganic solvents and high temperatures. Nature uses bilayer membranes to orient reactions, but engineers can graft enzymes onto polymers. Similar points came up during the discussion. In particular, the capabilities of the Langmuir-Blodgett (L-B) technique for achieving some of the desired miniaturization and orientation effects was stressed. The L-B technique has been used to form biological types of arrays coupled to the metallic conductors required for contact with practical devices.

It is obvious that the topics represented here could have been covered, and frequently are in the contexts of other disciplines e.g., biochemistry, bioenergetics. What was special here, and perhaps even

unique, was an examination of these subjects in biology from the point of view of electrochemistry. The perspective of electrochemistry is particularly useful for considering biological problems involving charge movement (e.g., ion transport, energy transduction, and electrical excitation), and the valuable insights gained from this interaction suggest new approaches to the solutions of the major problems in these areas.

**END**

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